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AT HARVARD COLLEGE

Vol. 112, No. 5

THE COMPARATIVE BIOLOGY OF REPRODUCTION
IN THE WOOD-BORING ISOPOD CRUSTACEAN
LIMNORIA

BY ROBERT J. MENZIES



CAMBRIDGE, MASS., U. S. A.
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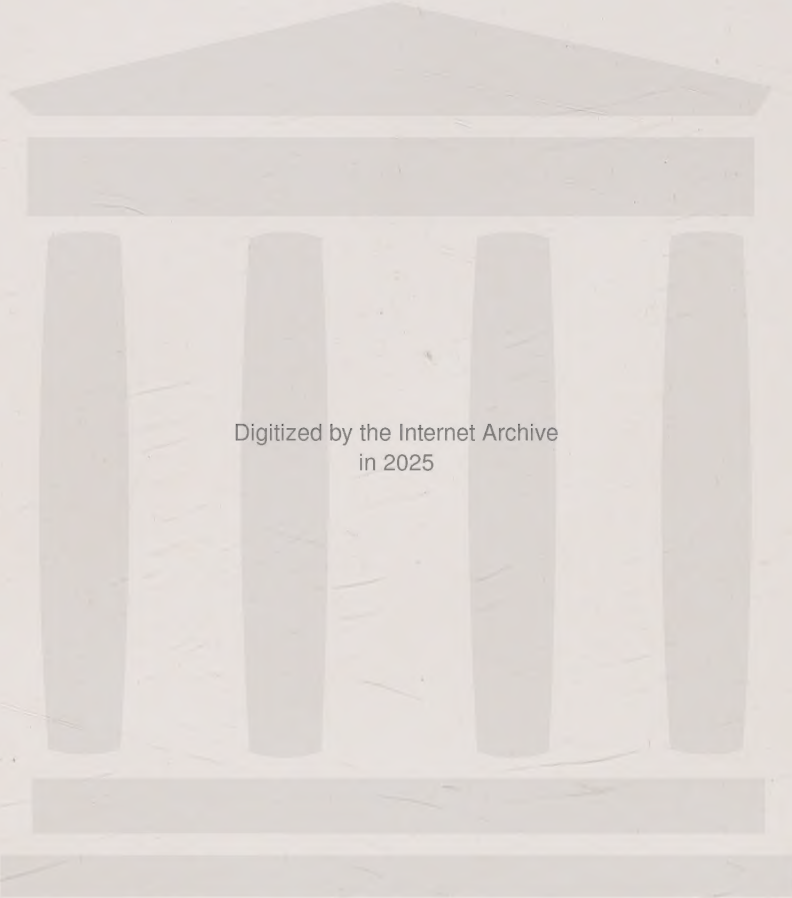
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No. 5 — *The Comparative Biology of Reproduction
in the Wood-Boring Isopod Crustacean Limnoria*¹

BY ROBERT J. MENZIES

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INTRODUCTION

A study of the biology of an organism necessarily involves the accumulation of data concerning many aspects of its anatomy and behavior. Knowledge about the way in which an animal reproduces, maintaining itself in its environment, is highly significant to an understanding of its biology. Here the external and internal anatomy of the marine pest *Limnoria* is described. Comparisons are made with other isopods in order that an idea may be had of the basic similarities and differences which *Limnoria* shows with the members of the crustacean order to which it belongs. Where possible, correlations are drawn between the structure of the reproductive system and the reproductive behavior of the animal.

This paper represents the results of part of a general study on the biology of *Limnoria* which was done under contract with the Office of Naval Research at the Scripps Institution of Oceanography in the Division of Marine Invertebrates in collaboration with Dr. Martin W. Johnson.

The first study of the internal anatomy of *Limnoria* was made by P. P. C. Hoek (1893, 97 pp., 7 pls.). Although written in Dutch and thus largely unavailable to American scientists, his work represents a most comprehensive study which has often been quoted and which forms the basis for a similar report by Kofoed and Miller (1927, pp. 306-332). Both investigations dealt with the entire organism, with only brief reference to the reproductive system. Various aspects of the reproductive behavior of *Limnoria* are also mentioned briefly by Coker (1923, pp. 95-100), Henderson (1924, p. 320), Johnson (1935, p. 428), Sømme (1940, p. 155) and Shiino (1950, p. 348), all of whom were mainly interested in the ecology of the animal.

MATERIALS AND METHODS

The species investigated was *Limnoria tripunctata* Menzies (Menzies, 1951, pp. 86-88) collected from San Diego harbor, California. Its reproductive system has been compared with those of *L. quadripunctata* Holthuis and *L. lignorum* (Rathke). No structural deviations of major importance were found, and it is therefore believed that the findings reported here are applicable to the majority of the species.

Living specimens, whole and decapitated, were fixed prior to sectioning, in formalin (10 per cent), Carnoy's fluid acid alcohol, formalin-alcohol-acetic acid (F.A.A.), and 70 per cent alcohol. Cytological details were best preserved in decapitated specimens fixed in F.A.A. Sections were made of paraffine-embedded samples at 7 μ and 10 μ . Three staining techniques were employed: Heidenhain's iron hematoxylin (counterstain eosin), Mallory's triple stain, and Harris' hematoxylin. The first gave the most generally useful results for this study.

COMPARATIVE MORPHOLOGY OF THE REPRODUCTIVE ORGANS

In common with all known isopods the body of *Limnoria* can be divided into three major regions (Fig. 1): the cephalon or head bearing the eyes, antennae, and mouth parts; the peraeon

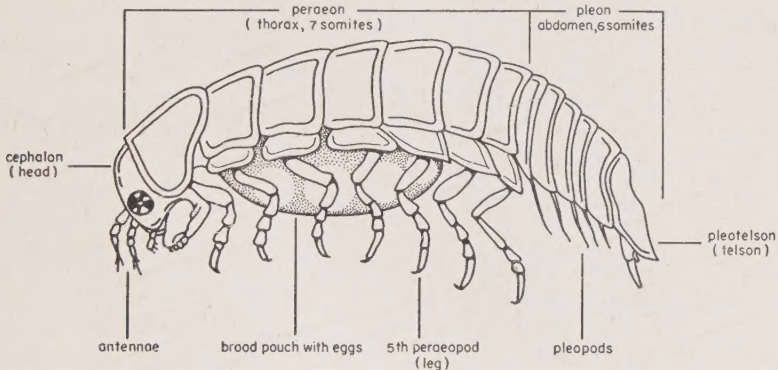


Fig. 1. Gravid female *Limnoria* (from G. O. Sars, 1897). Major regions of the body mentioned in text are noted. (The assistance of Mr. Robert Winsett, Scientific Illustrator, Publications Division, S.I.O., in the final preparation of Figures 1, 2, 5, 6 is particularly appreciated.)

or thorax bearing usually seven pairs of walking legs; and the pleon or abdomen bearing usually five pairs of swimming and respiratory appendages, the pleopods. The male and female gonads are contained within the peraeon. Their relationship to other major organs is shown in Figure 2. In *Limnoria* the sexes are separate but this is not true for several other isopods (p. 372). The body of an adult *Limnoria tripunctata* is roughly 2.50 mm. long and 0.60 mm. wide at the pleotelson.

Internal Anatomy

Female. The ovaries are paired organs located below and on either side of the tubular heart and above the intestine and digestive glands. Each ovary when mature extends from the second to the seventh (last) peraeonal somite. Both their size and extent are governed by the size and number of the developing ova. The

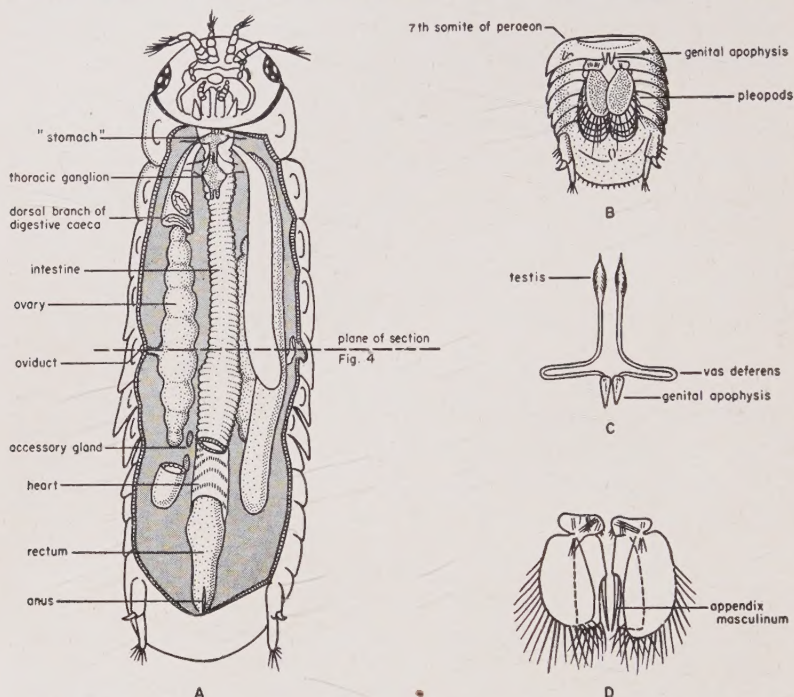


Fig. 2. Internal and external reproductive organs of *Limnoria*. A. Female, ventral view, body wall cut away. B. Male, pleon and seventh somite of peraeon, ventral view. C. Testes. D. Second pleopods of male. Figures B and D after G. O. Sars, 1897; A and C original.

immature ova (Fig. 3C) are about 0.045 mm. along the long axis and have a characteristic uniform cytoplasm when preserved, and a discrete nucleus. Yolk globules are not differentiated. In contrast, the mature ova (Fig. 4) are large oblong yolk-filled cells with a length of 0.30 mm. and a cross-sectional diameter of 0.12

to 0.18 mm. The space occupied by the eggs at the middle of the body is equal to about 30-40 per cent of that of the expanded body cavity itself. The increased volume of the maturing eggs compresses the other internal organs to the extent that females in the stage prior to egg deposition frequently do not have food in the gut.

The oviduct, attached laterally to each ovary at about its middle, extends ventro-laterally to open at the base of the fifth pair of legs. Each oviduct is band-shaped with a length of 0.08 mm., a thickness of 0.01 mm., and a height (distance between ovary and orifice of oviduct) of 0.12 mm.

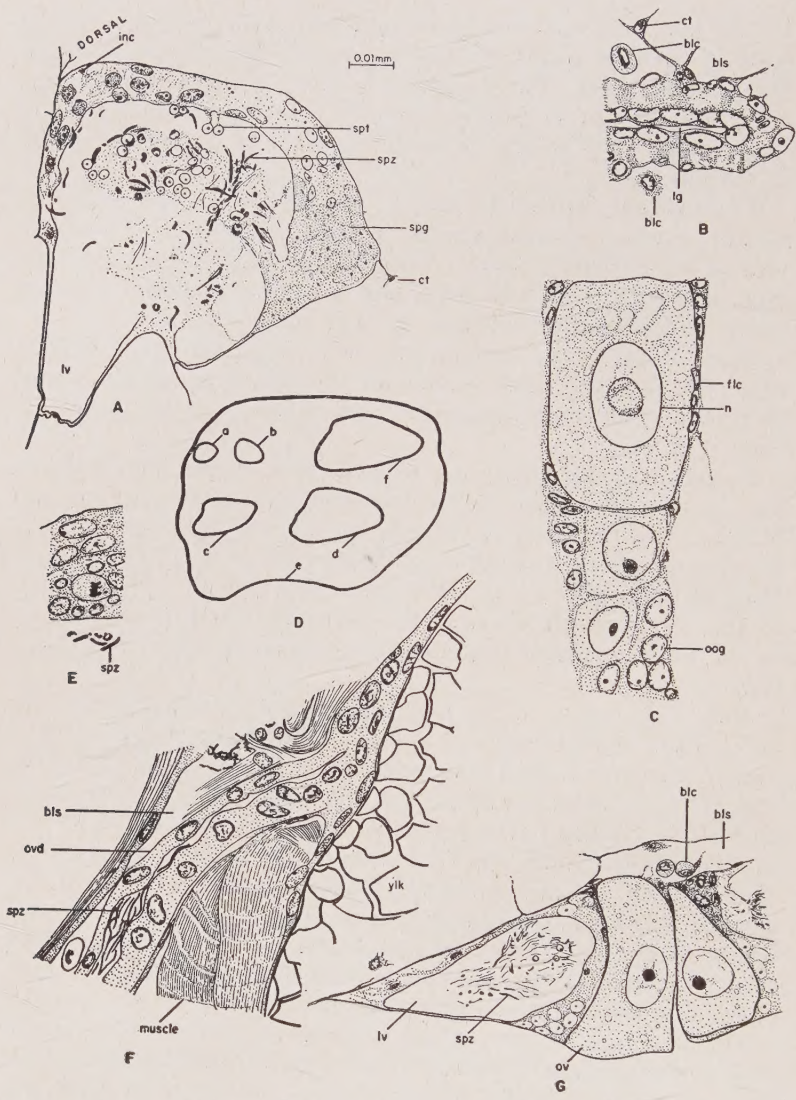
Much of the tissue surrounding each ovum consists of follicle cells (*flc*, Fig. 3C). The oogonia are distributed along the lateral margin of each maturing ovum and at the ends of the ovary (*oog*, Fig. 3C).

Posterior to each ovary are located two elongate glands which have a narrow lumen (called accessory glands by Kofoed and Miller). The function of these is not known. Dorsally they are in contact with the pericardium. Their cells (Fig. 3B) are unlike those of the ovary or any other organ in *Limnoria*. The location of the accessory glands near the heart and their presence only in the female suggest that they might have an endocrine function.

Male. The testes of the male are similarly paired (Figs. 2C, 3A). Each testis consists of a single lobe of approximately 0.08 mm. in diameter and 0.1 mm. in length, located between the fourth and fifth peraeonal somites, below and on either side of the heart and not in the last (7th) peraeonal somite as maintained by Kofoed and Miller (1927, *op cit.*).¹ A vas deferens proceeds from the testis to the seventh peraeonal somite. Here, as is usual for isopods, it turns medially and extends along the ventral body wall to one of the paired genital apophyses. The genital apophyses (=penis) consists of a pair of movable skin folds on either side of the midline of the seventh peraeonal somite.

The structure of the testis is unusual in having only a single lobe. Cytologically, spermatogenesis and spermiogenesis (Fig. 3A) are easily traced. Spermatogonia consist of rectangular shaped cells of uniform consistency and with an evident nucleo-

¹ Kofoed and Miller's error might be due to the mistranslation of Hoek's (*op. cit.*, p. 33) statement that the testes were located in the last body segments: "De testes nemen in de laatste borstsegmenten . . ."



lus. Primary spermatocytes have a greatly swollen nucleus and at the metaphase show tightly compacted tetrads and evident centrosomes. The nuclei of secondary spermatocytes are about one-half the size of those of primaries and have peripheral chromatin granules and an evident nucleolus. Spermatids show a variety of structure during their maturation to sperms. The nuclei of early spermatids have a granular nucleoplasm and apparently two nucleoli. Those in a later stage are much smaller with a clear nucleoplasm and two marked nucleoli. Mature sperm have a cap (head?), an elongate midpiece, and a tail piece which is at least twice the length of the midpiece. The precise length of the sperm tail was not determined. Various parts of the testis appeared to be in different phases of development. Morphologically mature sperm, however, were found in the lumen throughout the length of the testis. A hyaline membrane covered the testis and formed the wall of the vasa deferentia. It was associated with minute nuclei of the connective tissue outside the testis and appeared to have been secreted by those cells. Some small nuclei found inside the testis are considered to belong to interstitial cells.

It is common among isopods for sperm to mature together in compact batches (spermatophores). This bunching of sperm was not observed, however, to occur in *Limnoria*, and no spermatophore seems to be produced. Except for this deviation, the

Fig. 3. Sections of reproductive organs of *Limnoria* A-C, E-F. A. Cross section through testis. B. Longitudinal section through part of an ovarian accessory organ. C. Longitudinal section through immature ovary. E. Part of a cross section through testis showing large primary spermatocytes and smaller secondary spermatocytes. F. Section through oviduct of fertilized female; note sperm in oviduct. *Abbreviations:* *blc*, bloodcell; *bls*, blood sinus; *ct*, connective tissue; *inc*, interstitial cell; *flec*, follicle cell; *lg*, lumen of accessory gland; *lv*, lumen of vas deferens; *n*, nucleus; *oog*, oogonium; *ov*, ovum; *ovd*, oviduct; *spg*, spermatogonia; *spt*, spermatid; *spz*, spermatozoa; *yik*, yolk.

D. Outlines of second pair oostegites of *L. quadripunctata*, all drawn to same scale. Widths of pleotelsons of specimens in millimeters are: *a*, 0.59, *b*, 0.65, *c*, 0.70, *d*, 0.77, *e*, 0.77, *f*, 0.88.

Sections all drawn to about the same scale as indicated in figure A; all drawings were done with the aid of a camera lucida.

testis cytologically appeared much like those described for other isopods.

A sperm storage organ or seminal receptacle has been discovered in many of the female isopods whose internal anatomy has been studied. This includes *Trichoniscus*, *Asellus*, and *Jaera* (Fig. 5). In *Jaera* and *Asellus* the seminal receptacle consists only of a swollen part of the oviduct, but in *Trichoniscus* a separate pouch is present. The genus *Jaera* shows an apparently unusual modification in also having a dorsally-opening vagina. Female *Limnoria* appear to lack any sperm storage organs and in this regard are apparently similar to *Sphaeroma*.

External Anatomy

The secondary sex characteristics of the female consist of leaf-like plates (oostegites) originating at the medial base of the coxal plate of legs two, three, four, and five; and of the paired openings of the oviducts medial to the base of each fifth leg. With the absence of a vagina in the genus, each of these openings might be considered a vulva. The vulvae were not found in immature females. Similarly the oviducts seemed to remain closed (without a lumen) until a short time prior to copulation.

The male secondary sex characteristics consist of the genital apophyses (Figs. 2C, 6) which were already mentioned and a stylus-like appendage (appendix masculinum) attached on the medial side of the endopod of each second pleopod (Fig. 2D).

The secondary sex characteristics of isopods show some remarkable variation. For example, oostegites do not appear in the suborder Gnathiidea (Monod, 1926, pp. 202-210), Fig. 5E, although the area of the ventral part of the body surrounding the genital atrium might be considered homologous. Oostegites, as indicated for *Limnoria*, are present in most members of the suborders, e.g. Anthuroidea, Flabellifera, Bopyroidea, Valvifera, Asellota and Oniscoidea. The movable plates which form the genital apophyses of *Limnoria* develop from simple swellings of the integument. Such swellings constitute the adult condition in *Cymothoa* (Fig. 6G). A fusion of these lobes into a single piece (ductus ejactulatorius) seems to have occurred in at least two suborders independently. Thus *Idothea* (Valvifera) has two plates, whereas *Synidotea* has one (Valvifera). *Ligidium* has two, while *Philoscia*

(Fig. 6C) has one (Oniscoidea). An appendix masculinum is present on the endopod of the second pleopods of males belonging to the suborder Flabellifera. Here its function is not known but it has been presumed to act as an intromittent organ in passing the sperm from the genital apophyses to the vulva (or equivalent opening) of the female. In the suborder Asellota (the genus *Asellus* perhaps being exceptional), the first two male pleopods combine as a functional penis. This is the case also in the suborder Oniscoidea (Vandel, 1925); however, the pleopods are of a structure quite different from those of the Asellota (Fig. 6D).

Within the suborder Flabellifera, each appendix masculinum consists only of a flattened part of the endopod of the second pleopod which originates by splitting off the endopod. In most genera it is within the realm of possibility that the appendix is long enough to reach the oviducal opening of the female; however, on the appendix masculinum of *Limnoria* the lack of any grooves, specialized bristles, or rugosities, in which sperm might be transferred or held prior to or during copulation, suggests that the appendix masculinum might not have such a function. On the other hand, the genital apophyses are generally so short and located so near the midline of the male that it is difficult to imagine that these might reach the vulvae of the female which in contrast are located at the base of the legs of the female, far from the midline. Copulation among the Flabellifera has not been observed in detail and, therefore, no solution to the above puzzle is offered.

SEX DETERMINATION

Sex Ratio

The sexes of *Limnoria* are found in about a 50/50 ratio when taken from piling populations (Station A,¹ mean ratio of males to mature females, 0.95, and Station B,² mean ratio of males to mature females, 1.06; see Table 1). It is probable that males and females are produced in that ratio; however, this needs further confirmation. The presence of sex chromosomes might be suggested for *Limnoria* and other marine isopods in view of the finding by Staiger and Bocquet (1954) of female heterogamety in the marine isopod *Jaera marina*.

¹ Located at the U.S. Navy Target Repair Base, Point Loma.

² Located near the San Diego landing of the Coronado Ferry.

TABLE 1

Ratio of males to females (those with oostegites) on piling.
Specimens were taken from heavily infested wood, each sample
averaging about 1 square inch in area.

	STATION	STATION
MONTH	A	B
September 1952	0.95	0.83
October 1952	1.05	1.19
November 1952	1.26	0.93
December 1952	1.26	—
January 1953	1.12	1.31
February 1953	0.65	1.00
March 1953	1.20	1.00
April 1953	0.44	1.19
May 1953	0.95	—
June 1953	1.00	—
July 1953	1.92	—
August 1953	0.65	—
MEAN	0.95	1.06
MEAN STA. A AND B		0.99

Ambisexual Individuals

Ambisexual animals, those having the primary and secondary characteristics of both sexes, were encountered rarely. Out of the 1423 sexually mature animals examined from thirteen migrant populations, they were found only twice; once in a sample having 39 specimens, and once in a sample having 137 specimens. Three were found out of a total of 657 specimens from piling; one sample of 91 animals had two while another of 30 animals had one. These individuals had the genital apophyses of the male as well as the immature or postgravid oostegites of the female.

Sections of one ambisexual individual (Fig. 3G) showed the presence of ovaries with immature ova as well as testes with mature sperm. No oviducts were found. Protandry is a characteristic feature of one family of the Flabellifera, the Cymothoidae, in which the young are functional males (with rudiments of ovarian tissue) and progressively become functional females. In the Sphaeromidae, rudimentary testes are attached to the ovaries of the female.

REPRODUCTIVE BEHAVIOR

Brood-Pouch Formation

The development of the oostegites prior to copulation and brood deposition is a gradual process with their size increasing as the animal grows (Fig. 3D). Copulation, fertilization, the production of the large oostegites of the fully formed brood pouch, and egg deposition into the pouch of *Limnoria* all appear to take place within a brief period of time (probably no more than two or three days). The release of the young from the brood pouch is followed by a molt of the female (several laboratory observations) and a reduction in size of the oostegites to their pregravid size (Fig. 3D). This was suspected earlier to be the case when females with small oostegites were found to have apparently recently released young in their burrows. It follows then that females with pregravid oostegites can be either virgin

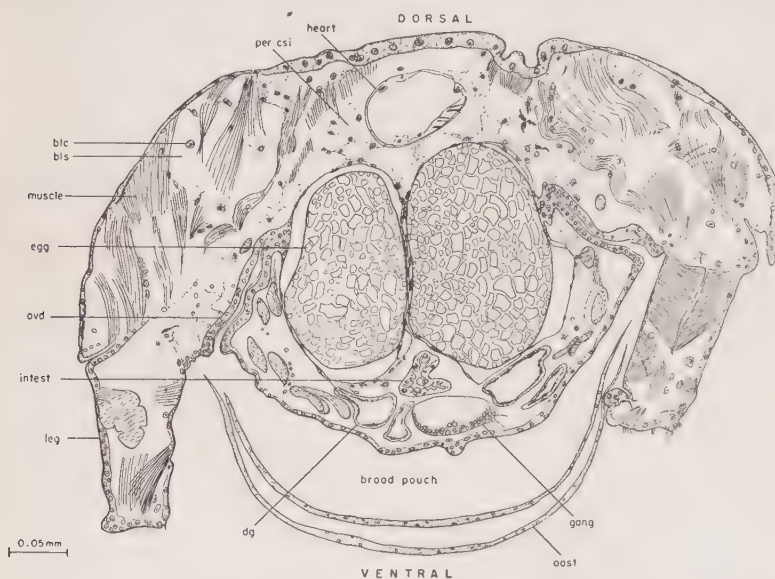


Fig. 4. Cross section through pre-gravid female *Limnoria* at oviduct. The animal had molted and the chitin was not heavily sclerotized at this stage. Note huge size of eggs and absence of food in intestine. Abbreviations, same as for Figure 3 with the following added: *dg*, digestive gland; *gang*, ganglion; *intest*, intestine; *oost*, oostegite; *per csi*, pericardial sinus.

females or females which have already produced one or more broods.

Incubation

Limnoria incubates its young within its external brood pouch (Fig. 5B). This mode of incubation is common to the majority of isopods, including the suborders Asellota, Oniscoidea, Valvifera, Anthuridea, Bopyroidea, Phreatoicoidea, and most of the

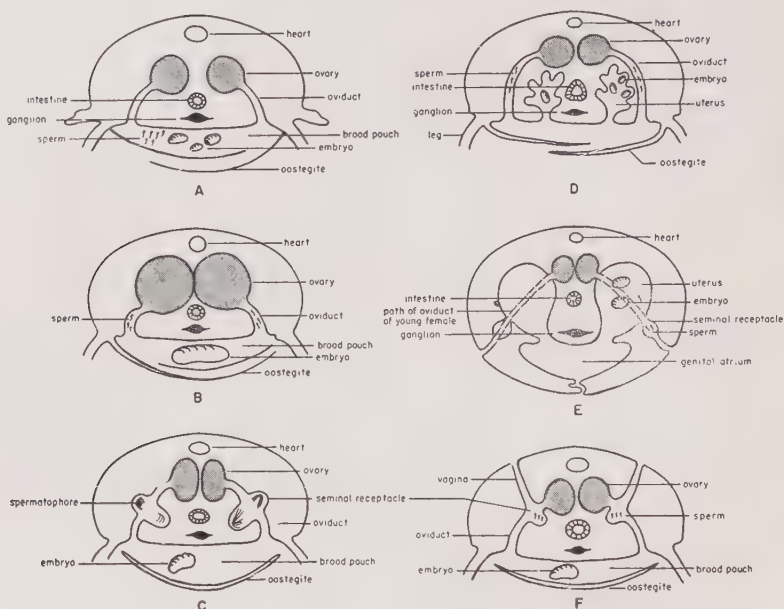


Fig. 5. Schematic diagrams of the female reproductive systems of the Isopoda. A. *Epipenaeon*, note external fertilization and absence of sperm storage organs. B. *Limnoria*, note absence of sperm storage organs. C. Oniscoid isopods, note presence of seminal receptacle and/or spermatophores. D. *Sphaeroma*, note presence of uterus and functionless "brood pouch." E. Adult *Paragnathia*, note absence of brood pouch and modifications undergone by female in development. F. *Jaera*, note presence of dorsal vagina. Figure A based on data from Hiraiwa (1936); B, original; C, from Vandel (1925); D, from Leichmann (1891-93); E, from Monod (1926), young based on figures given by Monod, adult from description by Monod; F, from Forsman (1944).

Flabellifera. The Gnathiidea and the Sphaeromidae (suborder Flabellifera) incubate their young "internally." In the Sphaeromidae the eggs are deposited into the brood pouch but then are taken into uteri which consist of paired invaginations of the ventral body wall. In the Gnathiidea no brood pouch is formed; instead the eggs are deposited into a genital atrium and then taken into the uteri which are reminiscent of those of the Sphaeromidae.

Pairing, Fertilization, and Burrow Construction

The occupation of a burrow usually by only a sexually mature male and female (plus any young produced by the pair) has been

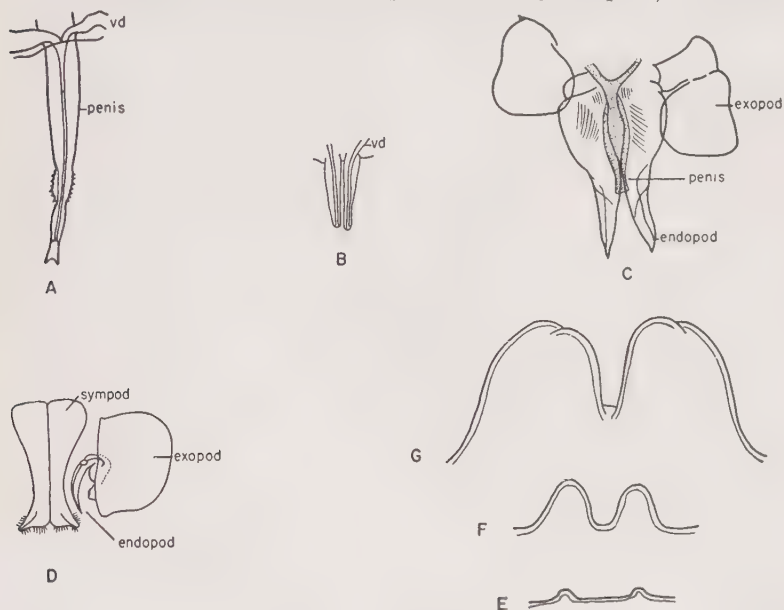


Fig. 6. Male reproductive organs. A. Penis of *Trichnoiscus dentiger* (after Vandel, 1925), B. Genital apophyses of *Ligidium hypnorum* (after Vandel, 1925), C. First pleopods and penis of *Philoscia muscorum* (after Vandel, 1925), D. First two pleopods of *Ianiropsis* sp. (after Menzies, 1952), E-G. Genital apophyses, E. Adult *Cirolana*, F. Young *Limnoria*, G. Adult *Cymothes*, all original. Scales of magnification variable. Abbreviations same as for other figures, *vd*, vas deferens.

TABLE 2

A comparison of the length of burrows occupied by single or by paired animals on test blocks of one- and two-month exposure at the U.S. Naval Target Repair Base, San Diego harbor, California (1953).

Burrow Length in mm.	No. Burrows with Single Animals		No. Burrows with Paired Animals	
	ONE-MONTH	TWO-MONTH	ONE-MONTH	TWO-MONTH
1.5	3	3	0	0
3.5	42	13	2	0
5.5	11	10	16	2
7.5	1*	6	12	8
9.5		1*	24	16
11.5			2*	14
13.5				10
15.5				9
17.5				2
19.5				1
21.5				1
23.5				1*
Total	57	33	56	64
Mean				
Burrow Length	3.8±0.97	4.8±1.95	7.6±1.9	11.8±3.4

* Length of longest burrow.

frequently observed (Henderson [1924], Johnson [1935], Shiino [1950], etc.). Examination of burrows in test blocks revealed that a burrow generally had two animals only when long enough to fully enclose two specimens (around 5.5 mm. and longer). The proportion of paired animals was found to increase markedly as the length of burrows and the length of exposure to migrant populations increased. Test blocks of one-month exposure had about as many burrows occupied by one as occupied by two animals, while those of two-month exposure had twice as many paired organisms as single ones (Table 2). Organisms kept in the laboratory have been observed to pair with each other for periods exceeding ten months (one case) and four months (four cases); presumably a similar relationship between paired sexes can be expected to occur in nature. Accordingly, it appears

that the pairing of sexually mature animals within a burrow is an essential and consistent feature of their biology.

Copulation apparently occurs in the burrow. The sperm produced by the male are relatively few in number and these, as has been seen, are not combined into a spermatophore. Sperm have been found in the oviduct of the female and probably fertilize the eggs as they pass down the oviducts. The absence in the female of any seminal receptacle suggests that a copulation must precede the deposition of each brood. This is more strongly indicated by the observation that sperm were not found in the oviducts of females bearing embryos or in virgin or post-ovigerous females but only in those with ripe ovaries which were molting, prior to egg deposition (Fig. 3F). In the terrestrial isopods which have been extensively studied in this regard and which bear a seminal receptacle, a single copulation has been found sufficient for the production of two broods (*Armadillidium*, Howard, 1940, p. 84). Heeley (1941, pp. 136-137) reports viable sperm to have been stored by isolated females of *Porcellio dilatatus*, *P. scaber* and *Oniscus asellus* for at least two successive year's brood. The sexes of *Porcellio* and *Armadillidium* show no tendency to pair and in the case of the latter, natural populations often have twice as many of one sex as the other (Howard, *op. cit.*). Oniscoid isopods (*Porcellio*, *Trichoniscus*, *Armadillidium*, etc.) usually have a seminal receptacle and the males produce many sperm which are united into a spermatophore (Schöbl, 1880; Vandel, 1925). In contrast, it is interesting to note that the diverse genera in which a long-term sexual pairing is a pronounced characteristic of the animals, no seminal receptacle is known to exist. These include the bopyrid, *Epipenaeon*, in which fertilization is apparently external (Hiraiwa, 1936, p. 108), the commensal and parasitic cymothoid, *Anilocra physoides* (Montalenti, 1941, pl. XIX), and *Sphaeroma* (Leichmann, 1891). It appears that pairing, a long-term association of members of the opposite sex, is correlated generally with an absence of sperm storage organs in the species which have been studied. In *Limnoria*, in view of its pairing habit, the production of sperm storage organs would teleologically seem superfluous.

There exists the remote possibility that parthenogenesis (which is known for several species of terrestrial isopods) might occur

in *Limnoria*. Five females kept for one year without a male failed to produce any brood, whereas seven paired animals produced one to two broods during that time. Hence parthenogenesis by *Limnoria* is considered unlikely.

TABLE 3

Size of broods (egg number) produced by various isopods

Classification	Species	Reference	Brood Size
ONISCOIDEA			
Ligiidae	<i>Ligia oceanica</i>	Vandel, 1925	25
	<i>Ligidium hypnorum</i>	“ “	3-17
Trichoniscidae	<i>Trichoniscus provisorius</i>	“ “	3-19 (7-11 av.)
	<i>Trichoniscus flavis</i>	“ “	6-18 (12 av.)
Oniscidae	<i>Tracheoniscus rathkei</i>	“ “	63
	<i>Porcellio monticola</i>	“ “	73
	<i>Porcellio scaber</i>	“ “	88
	<i>Oniscus asellus</i>	“ “	74
Armadillidiidae	<i>Armadillidium vulgare</i>	“ “	up to 200
FLABELLIFERA			
Cymothoidae	<i>Anilocra physodes</i>	Legrand, 1952	100-350
	<i>Livoneca convexa</i>	Original	67-130
Limnoriidae	<i>Limnoria</i> spp. (4)	This paper, p. 380	1-32 (6-21 av.)
Sphaeromidae	<i>Sphaeroma rugicauda</i>	Leichmann, 1891-93	63
GNATHIIDEA			
	<i>Paragnathia formica</i>	Monod, 1926	100+ (in ovary)
ASELLOTA			
Janiridae	<i>Jaera albifrons</i>	Forsman, 1944	5-60
	<i>Desmosoma</i> spp. (3)	Hult, 1941	8, 12, 16 (av.)
	<i>Ilyarachna</i> sp.	“ “	64
Munnopsidae	<i>Munnopsis typica</i>	“ “	32 (greatest number)
ANTHURIDEA	No data available		
BOPYROIDEA			
Bopyridae	<i>Epipenaeon</i>	Hiraiwa, 1936	2000+ (“several thousand”)
VALVIFERA			
Idotheidae	<i>Idothea</i> (<i>P.</i>) <i>resecata</i>	Original	46 (29-65)

Size of Brood

The size of the broods produced by *Limnoria* is comparatively small and variable both with regards to species and differing populations of the same species.

Isopods with pelagic young (bopyrid parasites) characteristically produce thousands of eggs per brood (Table 3). Other marine isopods belong to a category of marine organisms which produce fewer than a thousand young per brood (Thorson, 1950, p. 4). No free-living form is known to brood an excess of three hundred eggs.

The members of the genus *Limnoria* produce an average maximum of only 30 eggs per brood. Since few marine animals, even isopods, produce a smaller number of eggs, *Limnoria* may be classified with those marine animals which produce only a few eggs at one time. Except for the correlation between pelagic development of young and a high egg number, which is the rule among marine invertebrates, egg number among the isopods shows no pronounced phylogenetic correlation or pronounced correlation with size. Thus, within the suborder Oniscoidea, egg number per brood varies between 3 and 200, and in the suborder Flabellifera between 32 and 350. The size of the species is not a significant factor governing egg number because *Ligidium hypnorum*, a species much larger than *Limnoria*, does occasionally produce even fewer eggs than *Limnoria*. Similarly cymothoids which are generally much larger than many bopyrids produce several times fewer eggs. The number of eggs produced by an isopod seems more intimately related to the ecology and behavior of the species, and those factors influencing survival rate, than to other factors. One might suspect, in the burrow-producing *Limnoria*, that the survival rate of the young is very high in view of the animal's small brood size.

To judge from the available data on brood size (Table 4), it appears that the different species of *Limnoria* have differing brood sizes. Thus *L. lignorum* has been observed with a maximum of 35 eggs (mean 22), *L. quadripunctata* with 17 eggs at a maximum (9.5 mean), *L. tripunctata* with a maximum of 22 eggs (ca. 4-10 on an average), and finally *L. andrewsi* with a maximum of only 6 eggs. Here it is interesting to note that *L. lignorum*, a boreal species, has the greatest number; whereas,

L. andrewsi, a tropical species, has the least.

The evidence thus far assembled does not conclusively suggest, as has been the observed case for many organisms, that younger specimens (smaller) produce fewer eggs than larger specimens. In the species *Limnoria lignorum* (Rathke), Somme (1940) reported that broods produced during the summer were on an average larger by 10-12 eggs than autumn broods. Coker (1923)

TABLE 4

Number of eggs per brood in several species of *Limnoria*

Species	Kind of Distribution	Reference	Locality	Egg Number	
				Mean	Range
<i>L. lignorum</i> (Rathke)	Arctic-Boreal	Henderson (1924)	St. Andrews, Bay of Fundy	21.7	14-32
		Johnson (1935)	Friday Harbor, Washington	—	10-23
		Somme (1940)			
		a. summer broods	Flødøvig, Nor.	20-30	20-35
		b. autumn broods			10-20
<i>L. quadri- punctata</i> Holthuis	Temperate	Kofoed and Miller (1927)	San Francisco Bay, Calif.	9.5	1-17
<i>L. tri- punctata</i> Menzies	Temperate	Coker (1923)	Beaufort, N.C.		
		piling populations			
		a. early spring broods		4.2-6.6	1-12
	and	b. summer broods and fall		1.5-4.0	1-9
		c. winter broods		none	none
	Tropical	Shiino (1950)	Misaki, Japan	—	1-8
		ORIGINAL			
		a. piling populations	San Diego, Calif.	4.6±2.74	1-14
		b. Test-block population A	San Diego, Calif.	10.6±3.94	1-18
		c. Test-block population B	San Diego, Calif.	9.6±4.24	1-22
<i>L. andrewsi</i> Calman	Tropical	Shiino (1950)	Kominato, Japan	ca. 4-5	2-6

conversely found spring broods larger than summer and fall broods by several eggs (Table 4). He found no gravid females during the winter months. During the spring (April to May) the mean water temperature was near 17°C; during the summer and fall (June to September) it was near 26°C and never below 22°C. In contrast the 5-day averages of the winter water temperatures (December 13 to early March) varied between 11.3°C and 5.2°C. His data strongly suggest a close correlation between temperature and the production of eggs and a less strong correlation between temperature and brood size. Coker probably was working with *tripunctata* (not *L. lignorum*) which is the only species known to occur in North Carolina. As far as is now known *L. lignorum* does not occur south of Massachusetts on the Atlantic Coast.¹

Somme (*op. cit.*) found gravid females of *L. lignorum* throughout the year with maximal numbers occurring at temperatures averaging only 9°C and lesser numbers occurring when the temperatures averaged 3.9°C. During periods having similar water temperatures Coker (*op. cit.*) found no gravid females of *L. tripunctata*. Somme's evidence (*op. cit.*) indicated that little or no egg deposition occurred during the winter months but that the eggs deposited earlier were carried throughout the winter by the female.

These data indicate that an optimal range of temperature for the production of maximal-sized broods might occur in both species.

The presence of gravid females of *L. tripunctata* from piling in San Diego harbor and their absence from test blocks of one-month exposure during the winter months, when the temperature of the water was below 17°C, suggests a similar phenomenon; however, their presence on test blocks of two-months' exposure strongly indicates that egg deposition does not cease entirely as Coker found at Beaufort, but is only retarded under these less extreme conditions (Table 5).

In the case of *L. tripunctata* from San Diego harbor where temperatures near to Beaufort's spring temperatures (17°C) prevailed, it seems that factors besides temperature govern brood size. It has been found that migrant females (those from test

¹ Unpublished data.

TABLE 5
Seasonal occurrence of gravid females in piling and test-block samples in San Diego harbor.

MONTH	MEAN WATER TEMPERATURE (approximate)	PILING		TEST-BLOCK						TEMPERATURE AT COLLECTION	
				One-Month Exposure		Two-Month Exposure				TEMPERATURE AT COLLECTION	
				Per cent Gravid Females	Total Mature Females Examined	Per cent Gravid Females	Total Mature Females Examined	Per cent Gravid Females	Total Mature Females Examined		
September 1952	17.8	52	63	10	78					**17.4	
October 1952	18.0	27	22	36	25	33	**33			**18.8	
November 1952	16.9	22	37	8	12					**16.9	
December 1952	15.1	5	20	*0	21	68	**22			**14.8	
January 1953	14.9	12	25	*0	6					**14.0	
February 1953	14.9	25	20	*0	14	72	**22			**15.4	
March 1953	15.3	21	19	*8	13						
April 1953	14.1	32	38	*3	34	54	13				
May 1953	16.6	11	19	29	42						
June 1953	18.2	30	47	10	40	67	12				
July 1953	19.7	15	13	7	27						
August 1953	18.6	50	46	41	27	73	15				

* Sum of animals from three blocks, others from one block only.
 ** Year 1953 to 1954, not 1952 to 1953 as are all others.

blocks) produce an average of 5 more eggs per pouch than non-migrants (those from piling populations). The variable factor which correlates best here is differences in population density. The mean number of animals per square inch on test blocks of one-month exposure (Station A) from the top and bottom surfaces of the blocks for one year was 3.9.¹ The maximum density encountered (August 13, 1953) on those surfaces was 60 per sq. in. In contrast the density of piling populations (also sampled monthly at Station A) varied between 142 (lowest figure) and 620 (highest figure) animals per square inch (Table 6). A similar relationship between population density and fecundity has been indicated by Park (1939) for the flower beetle *Tribolium confusum*. It is equally difficult in the case of *Limnoria* to prove the *modus operandi* of a population density factor in lowering the fecundity of a population.

The fact that different species have differing brood sizes is of considerable interest indicating that perhaps the ecological factors associated with the reproductive capabilities of the various species are also different. Differences may be found in the frequency with which the broods are produced but this is an item about which no data are available at present.

TABLE 6

Egg number per brood of females from a piling population (presumably nonmigrant) and a test-block population (migrant) compared. Data collected at monthly intervals (1952-53) for twelve-thirteen months in San Diego harbor at two widely separated stations.

Location	Number of Gravid Females Examined	Mean Number of Eggs	σ $\pm$	Range
Piling, Sta. A.	307	4.66	2.70	1-14
Test-block, Sta. A.	105	10.06	3.94	1-18
Test-block, Sta. B.	113	9.63	4.27	1-22

¹ An empirically determined ratio of 1.5 animals times the number of burrows has been used here to estimate the total population of each test-block.

DISCUSSION

From this study it is apparent that the reproductive system of *Limnoria*, except for the fact that fertilization is internal, is a simple one with none of the specializations such as a vagina, spermatophore, seminal receptacle, or "internal" uterus which are known for many other isopods. Correlated with this simplicity of structure is the tendency of an adult of each sex to occupy a single burrow for long periods of time. This latter phenomenon suggests that *Limnoria* shows an elementary social life, with the paired animals working together in the construction and maintenance of a burrow and the production of the young.

The observation that the size of the brood of the various species is different, with the more tropical species having a fewer number of eggs than the species living in colder water, suggests that the rate at which broods are produced might be different with the different species. The fact that the rate of production of broods is dependent upon the environment inhabited by a species (as indicated by the data given here and earlier by Coker [1923] and Sømme [1940]) strongly suggests that the rate of brood production is not constant. These observations lead to a method for the evaluation of one hypothesis as to the cause of migration by *Limnoria*, namely overcrowding of the burrows. Once production rates for species in various localities and during various seasons are determined, these may be compared with the migratory picture, and a relationship based on more direct evidence can be established. The data indicate another important item relative to brood production, namely that both high and low temperatures encountered by a species can be associated with a lowering of the brood size. A rising environmental temperature then cannot be presumed always to be accompanied by a corresponding rise in the size of broods or in the rate of brood production.

Perhaps the most significant observation, one which should play a profound role in an estimation of the productivity of natural populations, is that the brood size of specimens from dense piling populations was found to be significantly lower than that of specimens from less dense migrant populations. Investigation of the factors associated with this observation should lead

to a better understanding of the population dynamics of this species as such investigations have done with other organisms for which this phenomenon has been reported.

SUMMARY

1. The internal and external anatomy and general histology of the reproductive organs of *Limnoria tripunctata* Menzies are described and compared with those of other isopods.

2. The male was found to have one-lobed testes in contrast to most isopods and was found not to produce spermatophores.

3. The female was found to have no sperm storage organs. Sperm were observed in the oviducts and it is believed that fertilization of the eggs occurs as they are passed down the oviducts into the brood pouch.

4. The absence of spermatophore production by the male, the absence of sperm storage organs in the female, and the finding of sperm in the female oviduct only prior to egg deposition suggest that a copulation must precede each brood.

5. The method of copulation was not observed. The simple unmodified structure of the appendix masculinum suggests that it is possibly not involved in sperm transfer.

6. A sex ratio of one male to one female is suggested from piling population analysis.

7. Ambisexual individuals were rarely encountered. These individuals had the genital apophyses of the male as well as the oostegites of the female externally and both testes and ovaries internally.

8. It was found that oostegites gradually increase in size until, within one molt, they suddenly increase greatly to form the brood plates of the brood pouch of the gravid female. A release of the young was observed to be accompanied by a molt of the female and a return of the oostegite to a pregravid size. Accordingly, it is indicated that females with pregravid oostegites can be either virgin females or females which have produced one or more broods.

9. The long-term pairing of a sexually mature male and a female in a burrow apparently is an essential and consistent feature of the biology of *Limnoria*. Specimens have been observed to remain paired as long as ten months.

10. The pairing behavior of *Limnoria* and that of several other isopods appears generally to be associated with an absence of any sperm storage mechanism.

11. The reproductive system of both sexes of *Limnoria* is simple in its organization as compared with that of other isopods.

12. Parthenogenesis does not appear to occur in *Limnoria*.

13. *Limnoria* belongs to a category of isopods which produce only a few eggs (average maximum of 30) per brood. A high survival rate of the young and/or a rapid rate of production is indicated.

14. Brood size was found to vary both with regards to the species and differing populations of one species.

15. The boreal species *L. lignorum* (Rathke) produces as many as 35 eggs per brood, whereas the tropical *L. andrewsi* Calman produces only 6 at a maximum. The temperate and temperate-tropical species *L. quadripunctata* Holthuis and *L. tripunctata* Menzies were found to produce broods between those extremes in size.

16. Under conditions of moderately low temperature (near 10°C) at Beaufort, N. C., *L. tripunctata* produced no broods. The brood production of the same species at San Diego during the winter months (temperature near 15°C) was retarded but did not cease entirely.

17. The data suggest that both high and low temperatures are associated with a lowering of brood size in *L. tripunctata* at Beaufort, N. C.

18. At San Diego harbor where seasonal temperature variations are moderate as compared with Beaufort, N. C., variation in brood size was found to correlate with population density, with animals from piling populations (high density) having fewer eggs per brood than animals from test-block populations (low population density).

19. A method of testing the hypothesis that overcrowding is the cause of migration in this species is proposed.

20. The following factors are indicated as important in estimations of the production rate of *Limnoria*: a) species involved, b) temperature and its effect on brood size and rate of brood production within a single species, c) population density and its associated factors as they affect brood size.

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